Cross-Species and Interassay Comparisons of Phytoestrogen Action

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This paper compiles animal and human data on the biologic effects and exposure levels of phytoestrogens in order to identify areas of research in which direct species comparisons can be made. *In vitro* and *in vivo* assays of phytoestrogen action and potency are reviewed and compared to actions, dose–response relationships, and estimates of exposure in human subjects. Binding studies show that the isoflavonoid phytoestrogens are high-affinity ligands for estrogen receptors (ERs), especially ERβ, but have lower potency in whole-cell assays, perhaps because of interactions with binding proteins. Many other enzymatic actions require concentrations higher than those normally seen in plasma. *In vivo* data show that phytoestrogens have a wide range of biologic effects at doses and plasma concentrations seen with normal human diets. Significant *in vivo* responses have been observed in animal and human tests for bone, breast, ovary, pituitary, vasculature, prostate, and serum lipids. The doses reported to be biologically active in humans (0.4–10 mg/kg body weight/day) are lower than the doses generally reported to be active in rodents (10–100 mg/kg body weight/day), although some studies have reported rodent responses at lower doses. However, available estimates of bioavailability and peak plasma levels in rodents and humans are more similar. Steroidogenesis and the hypothalamic–pituitary–gonadal axis appear to be important loci of phytoestrogen actions, but these inferences must be tentative because good dose–response data are not available for many end points. The similarity of reported proliferative and antiproliferative doses illustrates the need for fuller examination of dose–response relationships and multiple end points in assessing phytoestrogen actions. *Key words:* endocrine disruptors, estrogen agonists, estrogen antagonists, isoflavonoids, lignans, phytoestrogens. — *Environ Health Perspect* 109(suppl 1):5–20 (2001).
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Concerns about environmental substances with hormonal actions have generated scientific debate about the risks they pose for human and wildlife health. At issue are both the degree to which low potency may limit hormonal effects (*1*,*2*) and the difficulty of assigning potencies to compounds with life stage-, cell-, and gene-specific effects (*3*–*6*). Evaluating risk requires an accurate assessment of the relation between *in vitro* and animal tests of endocrine action and human responses. This task is daunting for many environmental substances, as the timing and quantity of human exposure are often uncertain, and suspected toxicity generally precludes their use in clinical trials.

The phytoestrogens, naturally occurring substances with estrogenic actions, provide an opportunity to examine these relationships more fully. Phytoestrogens exhibit a number of actions that have the potential to alter basic reproductive and developmental processes but also exhibit many potentially beneficial actions (*2*,*7*). Phytoestrogen actions and dose–response relationships have been assessed in both *in vitro* and *in vivo* studies and can be compared to human clinical and epidemiologic studies. Phytoestrogen exposures have been quantified for human subjects in a number of studies, and some pharmacokinetic data also are available.

This review article represents a first attempt to compile animal and human data on biologic effects and exposure levels of phytoestrogens in order to identify areas of research in which direct species comparisons can be made. *In vitro* and *in vivo* assays of phytoestrogen action and potency are reviewed and compared to actions, dose–response relationships, and estimates of exposure in human subjects.

Types of Phytoestrogens and Dietary Sources

A phytoestrogen is defined as any plant compound structurally and/or functionally similar to ovarian and placental estrogens and their active metabolites. This definition includes compounds with agonistic, partial agonistic, and antagonistic interactions with estrogen receptors (ERs) and other targets of estrogenic steroids involved in estrogen transport, synthesis, and metabolism. Functions affected by phytoestrogens include the regulation of ovarian cycles and estrus in female mammals and the promotion of growth, differentiation, and physiologic activities of the female genital tract, pituitary, breast, and many other organs and tissues in both sexes. Additional end points may include induction of RNA–protein synthesis and prolactin (PRL) secretion; prevention of bone loss; stimulation of hepatic production of sex hormone-binding globulin (SHBG), thyroid-binding globulin, plasminogen, and blood clotting factors VII–X; inhibition of antithrombin III and low-density lipoprotein (LDL) formation; and many others. Phytoestrogens differ in the combination of these actions they express and can be characterized as anticarcinogens, antioxidants, antiviral agents, and so forth. For example, genistein (GEN) can be characterized as a protein tyrosine kinase inhibitor, in contrast to its inactive chemical analogs daidzein (DAI) and chrysin. The latter phytoestrogens are equal or more potent inhibitors of steroid-metabolizing enzymes such as aromatase and 17-hydroxysteroid dehydrogenase (*8*,*9*).

The two major classes of phytoestrogens are lignans and isoflavonoids (Table 1). Some structurally related flavonoids also exhibit estrogenic properties. Lignans are minor components of cell walls and fibers of seeds, fruits, berries, vegetables, grains, and nuts. The isoflavonoids are prominent in legumes, especially soybeans, but detectable levels occur in whole-grain products, potatoes, fruits, vegetables, and alcoholic beverages (*9*,*10*), and in cows' milk, meat, and even fish grown with soy-containing food (*11*). The isoflavonoids are divided into three major classes: isoflavones, isoflavans, and coumestans (Table 1). The best-known isoflavones are GEN and DAI, which are formed from the plant precursors formononetin (FRM) and biochanin A, respectively. The most significant isoflavan is equol (EQL) (Table 2), a metabolite of DAI. Coumestrol (COM) is the best-known coumestan and the isoflavonoid with the highest estrogenic potency (*12*). The mycoestrogens are estrogenic fungal products that are not intrinsic components of plants but are found in pasture grasses and legumes infected by the fungal genus *Fusarium*.

Phytoestrogen Actions

Estrogen Receptor Affinity and Activation

Estrogen receptor binding is the best-documented action of phytoestrogens.

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Table 1. Major types and families of phytoestrogens.

Flavonoids
Isoflavones
DAI
Dihydrodaidzein
Formononetin
GEN
Biochanin A
Ononin
Prunetin
Isoflavanones
0-Desmethylangolensin
Isoflavans
Equol
Equol diacetate
Coumestans
Coumestrol
Coumestrol diacetate
4-Methoxycoumestrol
Hydroxychalcones
Phloretin
Isoliquiritigenin
4,4´-Dihydroxychalcone
Flavones
Flavone
Apigenin
7-Hydroxyflavone
4´,5-Dihydroxyflavone
Flavone acetic acid
4´,6-Dihydroxyflavone
7,8-Dihydroxyflavone
Chrysin
Tangeretin
Flavonols
Kaemperide
Kaemperfol
Quercetin
Flavanones
4´,7-Dihydroxyflavanone
Naringenin
Lignans
Enterolactone
Enterodiol
Matairesinol
Secoisolariciresinol
Resorcylic acid lactones
Mycoestrogens
Zearalenone
α-Zearalenol
α-Zearalanol (zearanol)
β-Zearalenol
β-Zearalanol

Numerous studies have shown that the isoflavonoids and lignans, as well as the fungal estrogens, compete effectively with estradiol (E_2) for binding to ER-rich cells or cytosol (Table 2). Interpretation of these data is complicated by the recent discovery that ERs are divided into two distinct subtypes: the originally sequenced ER, ERα, and a newly discovered variant, ERβ, with 95% homology in the DNA-binding region but only 55% homology in the ligand-binding domain (*12*). Binding studies using the purified receptor proteins show that the isoflavonoids COM and GEN are very active competitors for both receptor subtypes, but bind more avidly to the beta than to the alpha subtype (Table 2). COM has a 2-fold higher affinity for ERβ than for ERα, whereas GEN has a 30-fold higher affinity for ERβ (*12*). A recent study using human hepatoma cells has now shown that DAI also binds to ERβ with a higher affinity than ERα (*13*). This differential affinity is likely to be of functional significance, as the two receptor subtypes have different distributions across estrogen-responsive tissues (*12,14–16*) and during development (*17*). Moreover, as none of the common screening tests for estrogens such as ligand competition assays using rodent uterine cytosol or cell proliferation assays using breast cancer cells [e.g., E-SCREEN (*18*)] or recombinant cells containing ERα and an estrogen-responsive reporter gene [e.g., recombinant yeast cell bioassay (*18*)] include significant concentrations of ERβ (*18,19*), phytoestrogen potency may be underestimated.

Recombinant assays provide evidence that phytoestrogens not only bind to ERs but also initiate gene transcription. These assays are based on recombinant yeast cells containing an ER and one or more estrogen response elements (EREs) linked to genes for reporter proteins like β-galactosidase (GAL) (*20*), chloramphenicol acetyltransferase (CAT) (*20*), or human placental alkaline phosphatase (*21*). These assays have demonstrated that isoflavones, coumestans, and mycoestrogens all induce ERE-linked reporter proteins (Table 2). Most of these assays are based on ERα, but a recent study has shown that GEN activates reporter gene expression through both ERα and ERβ (*21*). However, in spite of GEN's higher affinity for ERβ, it may be a more effective inducer of ERα-mediated actions. Although GEN was slightly more potent via ERβ-linked reporters, it was less efficacious, displaying only partial agonism via ERβ but full agonism via ERα (*21*). Phytoestrogens can also alter the expression of receptors for hormones other than estrogen, including receptors for progesterone (PR), oxytocin (OTR), and testosterone (AR). In cell culture assays GEN induces PR expression at doses 1,000-fold lower than the concentration required to act as a tyrosine kinase inhibitor (*22*). GEN also acts as an antiandrogen in a dose-dependent fashion in a breast cancer cell line (*23*).

Perhaps the most compelling data come from *in vivo* studies using oral doses of phytoestrogens. Oral doses of DAI too low to be uterotropic significantly reduced mRNA expression of AR and ER in the rat uterus while upregulating the expression of complement 3, a gene known to be sensitive to estrogen, in a dose-dependent fashion (*24*). A 0.01% COM diet also failed to significantly alter uterine weight in female rats but significantly decreased OTR expression in the hypothalamus (*25*). These studies suggest that phytoestrogen can have a significant impact at the molecular level at doses too low to produce gross physiologic changes.

Postreceptor Activation

Some isoflavones and flavones inhibit protein tyrosine kinases (*26,27*), which play important roles in cell signal transduction and the regulation of the cell cycle (Table 3). GEN is the most potent inhibitor. GEN can also inhibit DNA topoisomerases I and II, enzymes essential for DNA replication (*28,29*), and is the most powerful antioxidant of the phytoestrogens. It also has the ability to increase the activities of antioxidant enzymes as well as directly inhibit hydrogen peroxide production.

Hormone Synthesis

Phytoestrogens have the potential to affect steroid biosynthesis and metabolism through a number of pathways (Table 3). A variety of *in vitro* assays have now shown that isoflavones and lignans are inhibitors of aromatase (*30,31*) and 5α-reductase (*32*). A number of phytoestrogens also inhibit 17β-hydroxysteroid dehydrogenase Type I (*33*).

However, these effects may not be as pronounced *in vivo*. Male rats fed a diet containing mulitple phytoestrogens (224 µg/g DAI, 319 µg/g GEN, and 39.5 µg/g glycitin) for 70 days showed no change in hypothalamic aromatase activity, even though isoflavone levels were 8 times higher in the brains of these animals than in the controls (*34*). Similarly, male rats fed a diet containing 200 µg/g phytoestrogens for 29 days also showed no significant changes in aromatase activity in either the amygdala or the preoptic area. However, significant changes were seen in both areas in the activity of 5α-reductase (*35*). The difference between the *in vitro* and *in vivo* data may stem from the significant role metabolism and the actions of binding proteins and second messenger systems play in the activity of phytoestrogens.

Cell Proliferation

Cell growth assays provide evidence that phytoestrogens can either enhance or suppress proliferation in estrogen-responsive cells, depending on their concentration and relative potency (RP) (Tables 4 and 5). COM, GEN, and α-zearalenol (α-ZEL) exhibit minimal antagonism of E_2 in cancer cell or recombinant yeast cell assays and sometimes even augment its action (Table 5). The weaker phytoestrogens, such as biochanin A and enterolactone (ENL), are antagonistic at higher levels, whereas flavonoids like phloretin (PHL), narigenin, kaempferol (KMP), apigenin (APG),

and β-ZEL have been reported to display triphasic activity, inhibiting estradiol at low and high concentrations and augmenting estrogen action at intermediate levels (*18*).

Hormone Transport

Interactions with binding proteins can have important effects on the RP of estrogens. Although serum albumins do not affect the bioavailability and activity of serum hormones, SHBG and α-fetoprotein differentially reduce sex-steroid availability, resulting in relatively greater bioavailability of estrogens that bind only weakly to these glycoproteins (*36*). Reports of phytoestrogen interactions with binding globulins are somewhat contradictory. An early study using dilute human serum reported phytoestrogen relative binding affinities of 14–27% (*37*), but affinities estimated in more recent studies using partially purifed SHBG range from 0.01 to 0.1% (*38*,*39*) (Table 6). The low affinity of isoflavonoids would be expected to result in enhanced bioavailability in the presence of SHBG, and in fact the effective free fraction of COM and GEN in human serum is 45–50%, whereas only 4% of E_2 is free (*40*). Addition of human serum to whole-cell binding assays raises the relative binding affinity of isoflavonoids 10-fold (*40*), but normal serum concentrations of SHBG (0.002 mg/mL) reduce the activity of COM and GEN in the yeast estrogen screen assay by only 5–10%, which is similar to the reduction of E_2 activity (5%) (*41*). However, pregnancy concentrations of human α-fetoprotein (40–160 mg/mL) reduce the activity of E_2, COM, and GEN by 50% (*41*).

Sequestering steroids is not the only action of binding globulins; binding globulins also contribute to hormone uptake by target cells (*42*,*43*–*45*). Cellular uptake of albumin- and SHBG-bound estrogens precedes their interaction with nuclear receptors and may directly affect the delivery of hormonal signals. Whereas free estrogens and the albumin-bound fraction are internalized into the cytoplasm, SHBG–estrogen complexes bind to membrane receptors, initiating nongenomic influences on cell metabolism through second messenger systems and priming effects on nuclear super-receptor complexes. ATP-binding cassette carriers within cell membranes also may expel estrogens out of cells, preventing their entry into the brain and other tissues. GEN binds to both the multidrug resistance protein (*46*) and P-glycoprotein (*47*), but only relatively high concentrations (200–400 μM) have been tested. These transport glycoproteins have low total capacity; ligands compete with one other for the binding sites, with the result dependent on the relative concentrations of binding

Table 2. Estimates of relative estrogenic potency of phytoestrogens.

Cell type	End point	Ref	DAI	FRM	GEN	BIA	DMA	EOL	COM	PHL	APG	KMP	QUC	NAR	IPR	ZLN	α-ZEL	α-ZAL	β-ZEL	β-ZAL
	ER-binding affinity: RP																			
Uterine epithelium	Uterine cytosol rat, sheep	(*165–167*)	0.1	0.01	1–2	00.01–0.04	0.05	0.2–0.4	11							1.4	27	17	0.430	3.4
Liver	Liver cytosol	(*168*)	0.01		0.1	0.001														
Breast cancer	MCF7 cells	(*104*,*149*)			0.1			0.1							None					
Breast cancer	MCF7 cells	(*142*)			2				13									18		
hER yeast	hER tranfected yeast cells	(*18*)			0.05	0.02			10	0.33	0.002	0.004		0.002			0.02		0.003	
PER-18 COS7	hER transfected COS7 cells	(*169*)	0.01			0.009														
Transcribed protein	hER-α protein	(*170*)			5				94											16.00
Transcribed protein	Rat ER-β protein	(*170*)			36				185											14.00
	Induced proteins: RP																			
Breast cancer	pS2: MCF7 cells	(*104*)			0.1			0.1				0.02	0.002			4.0				
Breast cancer	Exoprotein: MCF-7 cells	(*20*)	0.002	0.001	0.01	0.001			0.03											
hER transfected yeast	ERE-GAL	(*165*)	0.001	0.006	0.05	0.009		0.085	0.67							0.26	8.7	1.3	0.066	0.46
hER transfected yeast	ERE-GAL	(*41*)			0.01				0.1											
hER transfected Le42	ERE-CAT	(*20*)	0.003	0.0002	0.04	0.0005			0.06							2.5				
	Cell proliferation: RP																			
Breast cancer	MCF7 E-SCREEN	(*171*)							0.001							1	1			
Breast cancer	MCF7	(*172*)	0.0007	0.0004	0.08	0.005			0.11							0.85		47	0.043	
Breast cancer	MCF7	(*104*)			0.01							0.001	Inactive							
	In vitro *efficiency: RE*																			
hER transfected yeast	GAL-ERE induction	(*165*)	5.4	23	61	61		50	75							91	67	84	58.000	
Breast cancer	MCF7 proliferation	(*171*)							93							88	93			
	In vivo *estrogenicity: RP*																			
Uterine epithelium	Uterine growth sheep im	(*173*)			0.2	0.1			20											
Uterine epithelium	Uterine growth imm mice sc	(*165*)							0.02									0.026		
Uterine epithelium	Uterine growth imm mice oral	(*173*)	0.1	0.05	0.2	0.08	0.08	0.05	6											
Uterine epithelium	Uterine growth imm mice oral	(*174*)	0.008	0.0003	0.001	0.0005			0.04									0.026		
	RP to DES																			
Uterine epithelium	Uterine growth ovx rat oral	(*112*)			0.13															

Abbreviations: DMA, *O*-desmethylangolensin; hER, human estrogen receptor; im, intramuscular; imm, immature; NAR, naringenin; ovx, ovariectomized female; QUC, quercetin; RE, percent of response induced by maximal dose 17β-estradiol.

Table 3. Enzymes inhibited by phytoestrogens *in vitro*.

		ED_{50}									
End point	Ref	DAI	FRM	GEN	BIA	EQL	COM	APG	QUC	ENL	ZLN
Ovarian and placental aromatase	(*88–90*)	Inactive	Inactive	3.5 μM	2.2 μM	150–850 μM	Inactive	1.2 μM	12 μM	14 μM	
Preadipocyte aromatase	(*30,175*)				113 μM		17. μM			74 μM	
17β-Hydroxysteroid dehydrogenase	(*33*)			1.2 μM			0.1 μM				Inactive
5α-Reductase	(*32*)			35 μM							
In vitro angiogenesis	(*176*)			100–200 μM							
EGF TKE	(*177*)			2.6 μM							
EGF TKE (intact cells)	(*178*)			Inactive							
Protein kinase C	(*177*)			> 111 μM							
Phosphatidylinositol 3-kinase	(*179*)			Inactive				12 μM	10 μM		
Hydrogen peroxide production	(*180*)	150 μM		25 μM	Inactive						
Superoxide anion production	(*180*)	5 μM		1–2.5 μM	Inactive						
LDL oxidation	(*181*)	56 μM		13 μM	44 μM						
Microsomal lipid peroxidation	(*182*)	Inactive	Inactive	95 μM	53 μM	12 μM	5 μM				

TKE, tyrosine kinase-enzyme.

proteins and endogenous and exogenous estrogens (*9,48,49*). These interactions may play a role in the selectivity of the blood–brain barrier and other tissue–blood barriers and in absorption from the intestine. Although phytoestrogen influences on these processes are not well understood, their affinity for all these binding proteins and relatively high concentrations in plasma provide opportunities for significant effects on these mechanisms of hormone uptake.

In addition to binding competition, phytoestrogens might also influence estrogen availability by raising SHBG synthesis (*9*). The clinical evidence for this effect is mixed, with some studies demonstrating an increase in SHBG levels in postmenopausal women on a soy-based diet (*50*) and other studies showing no change in SHBG levels (*51–53*) or even a decrease (*54*) in SHBG on soy-based diets.

Phytoestrogen Potencies and Active Concentrations

Estimates of estrogenic potency vary across assays. Because recombinant cells often contain multiple copies of EREs, resulting in E_2 sensitivities as low as 0.1–7 pM, these assays can underestimate the *in vivo* concentrations required for estrogen action (*55*). Purified receptor preparations also provide unrealistic estimates of binding affinity caused by the absence of binding proteins. Cytosol and whole-cell assays provide more accurate estimates of potency, with effective concentrations in the picomolar to nanomolar range for E_2.

Generally, lower concentrations are required for ER-mediated events and for cell proliferation than for other types of responses. The range of estrogen concentrations required for stimulation of cell proliferation varies across assays, but in general, concentrations of 1–100 nM stimulate cell growth by isoflavonoids (Table 4). Relatively high concentrations, ranging from 5 to 100 μM, are generally required for suppression of proliferation (Table 5), and biphasic patterns are frequently seen, with proliferative actions occurring at nanomolar concentrations and inhibition of proliferation occurring at micromolar concentrations. There is evidence that lower concentrations of GEN are required to suppress environmental estrogen-stimulated cell proliferation if other phytoestrogens are also present. However, the concentrations required for these additive effects (25 μM) are still quite high, so it is unclear if the action would occur *in vivo* (*56*). Similarly, high micromolar levels are required for estrogen antagonism of cell proliferation, with minimal action by the most potent phytoestrogens.

Inhibition of tyrosine receptor kinases and other targets of phosphorylation occur at micromolar concentrations (Table 3). Micromolar concentrations are required for lignan and isoflavone binding to SHBG (5–50 μM) (*38*) and α-fetoprotein (K_d: 0.5–5 μM) (*57,58*). Concentrations required for inhibition of most steroid metabolizing enzymes are generally in the micromolar range, with the exception 17β-hydroxysteroid dehydrogenase type I, which is inhibited by isoflavonoids at concentrations ranging from 120 nM to 1 μM (*33,59*).

Phytoestrogen Exposure

Dietary Sources

The highest concentrations (1–3 μmol/g) of lignans are found in flaxseed (linseed) products (*60,61*), which are consumed by some Europeans. Good sources of lignans in the United States and Europe are pumpkin seed (20 mg/100 g), sunflower seed, cranberry, black tea, coffee, garlic, broccoli, brans, and peanuts (*62*). Green tea is a rich source of lignans and a popular beverage in many Asian countries (*63*).

Phytoestrogenic isoflavonoids are less prevalent than lignans. The highest concentrations are found in legumes, especially in soybeans and soybean-based products, which can contain as much as 0.2–1.6 mg of isoflavones per gram dry weight (*64*). The isoflavone contents of the basic food types are summarized in Table 7.

Plasma Concentrations

Humans. Phytoestrogen exposures vary substantially across human populations and individuals (Table 8). Differences across populations are related to dietary variation (*9,65*), whereas within-population variation smay reflect individual differences in the intestinal microflora, local differences in the phytoestrogen content of foods, and dietary effects on phytoestrogen absorption and metabolism (*11,66,67*). Asian diets are particularly high in soy, resulting in isoflavone consumption as high as 1 mg/kg body weight (bw)/day, whereas vegetarian diets are high in whole grains, vegetables, and legumes, resulting in higher consumption of lignans. These dietary exposures produce plasma isoflavone concentrations as high as 1 μM in Japanese men and women consuming a traditional diet. In Europe and North America, plasma concentrations are generally less than 0.07 μM for omnivores, whereas vegetarians may have levels as high as 0.4 μM isoflavones and 0.8 μM lignans. A recent study of Japanese infants indicates that isoflavonoids gain access to fetal tissues, resulting in concentrations in cord blood and amniotic fluid (0.2–0.3 μM) similar to maternal plasma concentrations (0.2 μM) (*68*).

Feeding trials provide more detailed data on phytoestrogen bioavailability in adults (Tables 9,10). Single soy meals providing isoflavone doses of 0.06–1.2 mg/kg (4–71 mg) produce peak plasma concentrations of 0.06–2.2 μM. Peak plasma concentrations of GEN and DAI are reached within 4–8 hr, with an absorption half-life of 1–3 hr. The excretion half-life ranges from 3 to 8 hr. DAI, with estimates ranging from 16–66%, appears to be more bioavailable than GEN (5–37%).

The highest human dietary exposures occur in infants fed a soy-based formula

Table 4. Estrogenic potency: *in vitro* range of concentrations for estrogenic actions.

End point	Ref	E_2	DAI	FRM	GEN	BIA	DMA	EQL	COM	APG	KMP	QUC	IPR	ENL	ZLN	α-ZEL	α-ZAL	β-ZEL	β-ZAL
ER binding																			
ERα protein	(*12*)	0.2 nM			4.2 nM				0.2 nM										1.3 nM
ERβ protein	(*12*)	0.1 nM			0.4 nM				0.1 nM										0.9 nM
Uterine cytosol	(*165,167*)	0.002 nM	1 μM	49 mM	100 nM	> 10 μM	2 μM	250 nM	20 nM						714 nM	4 nM	5.9 nM	232 nM	294 nM
Mammary cytosol	(*111*)			200 nM															
MCF7 cells	(*104,149*)	0.2 nM	61 nM		200 nM			200 nM			1.6 μM	20 μM	Inactive						
hER transfect COS7 cells	(*169*)	1 nM	60 μM			> 100 μM													
hER transfected yeast	(*18*)	1 nM			2 μM	6 μM			10 nM	44 μM	22 μM					6 μM		30 μM	
Induced proteins																			
pS2 in MCF7	(*104*)	0.2 nM	< 1 μM		200 nM			200 nM			1μM	10 μM		≥ 1 μM					
Exoprotein in MCF7	(*20*)	0.03 nM	2.5 μM	3 mM	300 nM	2.5 mM			100 nM						0.6–1 nM				
Cyclin D1 in MCF7	(*183*)	1 nM			500 nM										500 nM				
Prolactin	(*112*)	0.01 nM		10 mM				1 μM	100 nM					10 μM	10 μM				
Luciferase in MVLN	(*183*)	1 nM			100 nM										10 nM				
GAL in hER yeast	(*41*)	0.2 nM			100 nM				20 nM										
GAL in hER yeast	(*165*)	0.001–1nM			1 nM–10 μM				0.1–1,000 nM						0.1 nM–1 μm				
CAT in hER LeC9	(*20*)	0.2–30 nM	10–50 μM		1–50 μM				600–4,000 nM						50 nM				
CAT in hER FLG29.1	(*169*)	10 nM	> 1 μM										>1 μM						
Intracell signals																			
Cdk2 in MCF7	(*184*)	1 nM			1 μM										1 nM				
pRb105 in MCF7	(*184*)	1 nM			500 nM										500 nm				
Cell proliferation (Active range)																			
MCF7 cells	(*172*)	0.001 nM	0.01–100 μM		10 nM–0 μM	0.1–10 μM			10 nM–10 μM										
MCF7 cells	(*104,142*)	—	< 1 μM		1 nM–10 μM			0.01–10 μM	10 nM–1 μM		1–10 μM	Inactive		< 1 μM			10–100 nM		
MCF7 cells	(*185*)	0.5–1 nM												0.5–10 μM					
T47D cells	(*104*)	0.01–1 nM			10 nM–6 μM														
T47D cells	(*33*)	—			100 nM–1 μM	0.1–1 μM			0.01 nM–1 μM		Inactive	300 nM	Inactive			0.01 nM–1 μM			

Abbreviations: Cdk2: cylcin-dependent kinase 2; pRb105: retinoblastoma susceptibility gene product.

Table 5. *In vitro* concentrations for estrogen antagonism, augmentation, and antiproliferative actions.

Cell type	End point	Ref	DAI	GEN	BIA	DMA	EQL	COM	PHL	KMP	QUC	NAR	ENL	ZLN	α-ZEL
Brain capillary BBCE	Antiproliferative ED_{50}	(*176*)	90 μM	5 μM		50 μM	30 μM								
Breast MCF7	Antiproliferative	(*104,172*) (*108,185*)	Inactive	18–111 μM	50–100 μM		> 20 μM	50–100 μM		20 μM	.01–20 μM		50 μM		
	Inhibit 5 nM E_2	(*56*)		25 mM											
	Inhibit 1 nM E_2	(*185*)											1 mM		
	Inhibit 10 μM DDT	(*56*)		25 μM											
	Augment 0.3 μM DDT	(*184*)		1 μM										1 nM	
Breast T47D	Antiproliferative	(*104*)		10–20 μM											
	Inhibit 0.3 nM E_2			30–300 μM											
	Augment 0.1 pM E_1	(*33*)		1–100 nM				1–100 nM							
Breast MDA48	Antiproliferative	(*108*)		18–111 μM											
Prostate LNCAP	Antiproliferative	(*108*)		18–111 μM											
Prostate DV145	Antiproliferative	(*108*)		18–111 μM											
hER transfect yeast	Inhibit 1 nM E_2	(*18*)		Inactive	0.1–10 μM			Inactive	0.01–10 μM			1–100 pM			Inactive
	Inhibit 1 μM 4-OH-PCB				0.1–10 μM				> 1 μM			> 10 nM			
	Augment 250 nM COM				1 μM										

Abbreviations: BBCE, bovine brain capillary endothelial cells; 4-OH-PCB, 4-hydroxy-polychlorinated biphenyl.

Table 6. Phytoestrogen affinities for binding proteins.

End point	Ref	E_2	DAI	FRM	GEN	EQL	COM	KMP	QUC	NAR	ENL	END	ZLN	α-ZEL
Human serum RBA, $\%E_2$	(*37*)	100		24	27		14						5	21
SHBG affinity RBA, $\%E_2$	(*38*)		0		0.03	0.05					0.1	Inactive		
Human serum RBA, $\%E_2$	(*39*)	100	0		< 0.01		0							
Rat α-fetoprotein K_d, $\%E_2$	(*58*)	100	0.1		0.1	0.1		1	1	1				
Rat α-fetoprotein RBA, $\%E_2$	(*39*)	100	< 0.1		0		< 0.1							
SHBG comp ED_{50}	(*186*)	0.0031 μM			4.5 μM					1.9 μM				
SHBG comp ED_{50}	(*38*)	0.02 nM	> 100 μM		30 μM	20 μM					10 μM	> 100 μM		
Rat α-fetoprotein K_d	(*58*)	5 nM	5 μM		5 μM			0.5 μM	0.5 μM	0.5 μM				
Rat α-fetoprotein K_d	(*57*)	3 nM				6.7 μM					17 μM	22 μM		
P-glycoprotein minimum active	(*47*)				200 μM									
Multidrug resistance protein minimum active	(*46*)				400 μM									

Abbreviations: END, enterodiol; RBA, relative binding affinity; SHBG comp ED_{50}, SHBG competitive binding affinity, 50% estrogen displacement.

Table 7. Isoflavone and lignan content of human foods.

Food	GEN	DAI	Lignans
	mg/100 g		
Fruit			60–200
Vegetables	1–3	1–3	100–400
Cereals			100–700
Flaxseed			68,000
Soy products	20–1,100	20–900	900
Fava beans		100	
Indian bread root leaves	200		
Kudzu root		95	
Other nonsoy legumes	0–80	0–10	

Data from Aldercreutz and Mazur (*9*), Thompson et al. (*61*), Reinli and Block (*187*), and Kaufman et al. (*188*).

(SBF). SBF contains high concentrations of isoflavones (100–175 μM), significantly more than breast milk before (18–56 nM) or after (10–70 nM) soy consumption (*69*) or in bovine based-milk formula (*70,71*). An infant fed only SBF consumes approximately 6–11 mg/kg/day isoflavone, which is considerably more than the average for adults, even on a soy-rich Asian diet (0.3–1.2 mg/kg/day) (*72,73*). These high intakes result in markedly higher plasma concentrations (2.4–6.5 μM) in infants fed a soy milk diet than in infants fed either cows' milk formula (0.03 μM) or breast milk (0.02 μM) (*71,74*), plasma levels that are 10-fold higher than the average levels reported for Japanese women and equal to those seen at peak plasma levels following soy challenges in adults.

Animal models. Species differences in metabolism may influence bioactive doses. For example, relatively high oral doses are required to produce micromolar concentrations in plasma in sheep, whereas similar peak concentrations can be achieved in cattle and pigs with lower doses (Table 11). However, few studies have examined the pharmacokinetics of isoflavones in rodent models of estrogen action. Estimates of bioavailability of GEN in rats and mice (10–20%) are similar to values reported for humans (Tables 10,11); however, the doses tested in rodents are substantially higher than the doses tested in humans and use free GEN rather than the GEN glycosides present in the soy protein generally used in human trials. Peak plasma levels are achieved in rats within 2 hr and in mice within 3–30 min after oral dosage (Table 11). Single or repeated oral GEN doses of 20–45 mg/kg produce peak plasma concentrations of 2–11 μM in rats. Single oral doses of 50–200 mg/kg GEN produce peak free plasma concentrations of 1–4 μM in mice. These doses are considerably higher than those reported to produce micromolar plasma levels in humans, but data on plasma concentrations resulting from lower dietary doses in rodents will be required to determine whether these dose responses represent species differences.

Tissue Concentrations

Only a few studies have examined phytoestrogen concentrations in tissues and bodily fluids other than plasma and urine (Table 12). Single-dose treatment of female mice with 40 mg/kg/day subcutaneous (sc) FRM produced peak plasma concentrations of 9 μM in 2 hr and peak mammary tissue concentrations of 7 nmol/g in 4 hr, with mean half-lives of elimination of 2 and 2.5 hr, respectively (*75*). In rats, plasma levels of EQL increased from 91 to 450 nM 1 hr following a single sc injection of 4 mg/kg EQL, and the corresponding uterine concentrations were 0.4 and 4 nmol/g (*76*). Chronic oral doses of 8–40 mg/kg GEN resulted in reproductive tissue concentrations of 0.3–1.4 nmol/g (*77*). On a parts per million basis, total isoflavone levels in rodent plasma and mammary gland appear to be quite similar. However, the high proportion of GEN aglycone in reproductive tissues compared to serum suggests that some accumulation occurs (*77*).

A very small sampling of human breast milk (HBM) suggests that isoflavone concentrations are lower in breast milk than in plasma (*69,78*) (Table 12). On the other hand, a large sample of prostatic fluid concentrations indicates that isoflavone and lignan concentrations are higher on average than mean plasma levels (*79,80*) (Table 12). Plasma and prostatic fluid concentrations in individual men were well correlated for DAI (r_s = 0.7) but not for enterolactone (r_s = 0.2–0.4) (*80*), suggesting that isoflavones are concentrated in the prostate.

The most illustrative study to date used an oral dose of [^{14}C]GEN (4 mg/kg) in male and female rats (*81*). The highest concentration of radioactivity was found in the gut; significant levels of radioactivity were also found in a variety of other organs, particularly the liver and reproductive organs, but also the brain, heart, lungs, and kidneys. Interestingly, retention in the liver was sexually dimorphic, with females showing nearly 2.5 times the radioactivity as males 2 and 7 hr after the initial dose was given.

Exposure and Bioactivity

The data reviewed above indicate that total plasma levels of isoflavonoids and lignans in humans range from 10 to 400 nM, of which only about 10% (1–25 nM) is unconjugated (Table 8). Plasma concentrations as high as 2 μM total isoflavonoids may be achieved for a few hours after food consumption. Limited data for tissue concentrations indicate no more than nanomolar levels, even in tissues like the prostate where isoflavonoids may be concentrated relative to plasma. Therefore, overall it appears that average concentrations may be too low to induce most of the reported *in vitro* actions other than those mediated by ERs (Table 3). Moreover, current data suggest that agonism and cell proliferation are more likely than antagonism and suppression of cell proliferation. However, the latter predictions may be biased by the ERα-based systems from which they were drawn, and a different picture may emerge from assays based on ERβ.

In Vivo Actions

A variety of *in vivo* actions of phytoestrogens have been reported in animal experiments

Table 8. Mean plasma isoflavonoid and lignan concentrations in human populations consuming typical diets.

Locale	Diet	Sex/age	DAI (nM)	GEN (nM)	DMA (nM)	EQL (nM)	Total (nM)	ENL (nM)	END (nM)	MAT (nM)	Total (nM)	*n*	Ref
Total													
Spain	OM	M	1.3			0.4		3.9	0.4		4.3	50	(*80*)
United Kingdom	OM	M	8.2			0.6		3.9			3.9	36	(*80*)
Finland	OM	M	6.2	6.3	< 0.1	0.3	12.9					14	(*189*)
Canada	OM	M	3.4	8.2			11.6	13.9	1.9		15.0	10	(*190*)
Hong Kong	OM	M	31.3			3.8		6.2	1.7		7.9	53	(*80*)
Japan	OM	M	107.0	276.0	3.3	5.5	391.8					14	(*189*)
Finland	OM	F-PRE	4.2	4.9	0.1	0.8	10.0	28.5	1.4	0.0	314.9	14	(*191*)
Finland	VG	F-PRE	18.5	17.1	0.8	0.7	37.1	89.1	5.4	0.1	94.6	14	(*191*)
Finland	LV	F-PRE	41.5	29.7	1.8	1.0	74.0	752.7	65.6	1.9	820.2	3	(*191*)
Finland	OM	M	0.6	0.5	< 0.1	0.1	1.3					14	(*189*)
Japan	OM	F-PG					232						(*68*)
Japan	OM	F-PRE	72.5	206.1									(*192*)
Japan	OM	NCB					299						(*68*)
		NAF					223						
United States	HBM	Infant					3600						
	CMF						34						
	SBF						17						(*71,73*)
Free + sulfate													
Japan	OM	M	12.8	7.8	1.8	0.6	23.0					14	(*189*)
Finland	OM	F-PRE	0.7	0.7	0.0	0.0	1.4	4.9	0.2	0.0	290.1	14	(*191*)
Finland	VG	F-PRE	3.2	1.3	0.1	0.2	4.8	16.8	0.7	0.0	302.5	14	(*191*)
Finland	LV	F-PRE	6.5	1.1	0.6	0.3	8.4	203.6	17.2	0.8	221.6	3	(*191*)

Abbreviations: CMF, cows' milk formula; END, enterodiol; F, female; LV, lacto vegetarian; M, male; NAF, neonatal amniotic fluid; NCB, neonatal cord blood; OM, omnivorous; PG, pregnant; VG, vegetarian.

Table 9. Human plasma and urinary isoflavone concentrations following a single dietary dose.

Daidzein					Genistein					
Dose mg/day	Dose (mg/kg/day)	Peak plasma (nM)	Urine (μmol/day)	Ref	Dose (mg/day)	Dose (mg/kg/day)	Peak plasma (nM)	Urine (μmol/day)	*n*	Ref
0	0		3.8	(*193*)	0	0		1.2	12	(*193*)
0	0		3.8	(*194*)	0	0		1.1	12	(*194*)
0	0	3		(*190*)	0	0	8		10	(*190*)
0	0		0.3	(*66*)	0	0		0.2	17	(*66*)
0	0		0.2	(*53*)	0	0		1.8	21	(*53*)
3.9	0.06	60		(*78*)	4.4	0.07	100		2	(*78*)
7.9	0.1	500		(*78*)	8.9	0.14	350		2	(*78*)
15.7	0.3	1,400		(*78*)	17.8	0.3	1,300		2	(*78*)
21	0.4	1,400	51.8	(*195*)	19.3	0.3	740	3.6	12	(*196*)
21	0.4		3.6	(*66*)	30	0.5	1,300	14.4	7	(*195*)
24.7	0.4	790	19.0	(*196*)	34	0.6	1,400	19.2	7	(*195*)
26	0.4	1,500	59.0	(*195*)	37	0.6		11.5	40	(*53*)
27	0.4		22.2	(*53*)	38	0.6		8.4	12	(*194*)
27	0.4	424		(*197*)	36.2	0.6	1,070	14.3	12	(*196*)
	0.4	498		(*155*)	44	0.7		1.7	17	(*66*)
32	0.5		14.7	(*194*)	55.7	0.9	2,150	20	12	(*196*)
34	0.6		3.9	(*66*)		1.0	907			(*155*)
35.6	0.6	498		(*190*)	73	1.2		1.7	17	(*66*)
45.9	0.6	1,220	41.9	(*196*)	80	1.3	907		10	(*190*)
70.7	1.2	2,224	56.4	(*196*)						
80	1.3		14.7	(*193*)						

and human clinical studies. Although the majority of these actions are in the reproductive tract, there is evidence for effects on functions of the cardiovascular, skeletal, and central nervous systems.

Proliferation of Uterine and Vaginal Epithelium

Proliferation of the female reproductive tract is a classic test of estrogenicity that has been demonstrated for a number of phytoestrogens in a variety of animal species (Table 13). More variable results have been achieved using soy treatments. A soy-based diet providing 7 mg/kg/day GEN and 3 mg/kg/day DAI to ovariectomized rhesus macaques did not induce uterine growth or maturation of the vaginal epithelium, although they produced significant changes in cardiovascular risk factors (see below) (*82*). A similar diet also failed to alter uterine growth or vaginal cytology in ovariectomized rats (*83*).

More variable results have been obtained with postmenopausal women. Soy protein supplements that provided postmenopausal women with 0.7 mg/kg/day GEN and 2.1 mg/kg/day DAI over a 4-week period did not significantly alter the vaginal maturation index, although there was a trend for an increase in superficial cells (*84*). On the other hand, increases in the maturation index were observed in an Australian study during 6-week supplementation with a soy flour

(*85*) estimated to have provided similar isoflavone doses (e.g., about 1–2 mg/kg/day) (*84*). This study did not include a control group, however, so the results are difficult to separate from the normal waning of menopausal symptoms over time. It may be that the actual dietary content was higher, as another Australian study that provided the same supplement reported much higher urinary excretion (45 µmol/day) of DAI (*86*) than has been reported in other feeding studies (Table 8); however, the latter study did not observe any changes in vaginal cytology. Two more recent studies did not observe changes in vaginal cytology during soy supplementation (*50,87*).

Ovarian Cyclicity

Phytoestrogens could influence ovarian cycles through several pathways. Phytoestrogen interaction with ERs in ovarian granulosa cells might augment or inhibit follicular development, especially in light of the high affinity of isoflavones for ERβ. ERβ is abundant in granulosa cells, although the high intraovarian concentrations of E_2 may limit the potential for such effects. Phytoestrogen inhibition of ovarian aromatase (*88–90*) could reduce the availability of endogenous estrogen and limit follicular development. However, the very high concentrations required for inhibition by isoflavones (Table 3) make it unlikely that they would significantly influence estrogen production in the ovary. A more likely route for isoflavone influences on E_2 bioavailability would be via inhibition of 17-hydroxysteroid dehydrogenase type I, an effect that occurs at concentrations of 0.1–1 µM (*33,59*). In addition, phytoestrogens could augment or inhibit estrogen negative feedback by binding to ERs in the anterior pituitary or hypothalamus and indirectly alter ovarian steroidogenesis (see below).

Natural dietary exposures to phytoestrogens have been associated with cystic ovaries, irregular estrus, and anestrus in cattle (*91*), and reduced breeding success in California quail (*92*). Compromised follicular development (*91*) and reductions in luteal phase plasma progesterone and E_2, as well as shortened luteal phases, have been reported in cycling ewes whereas increases in estradiol and cortisol have been reported in pregnant ewes (*93,94*). Soy isoflavone diets providing doses of 1 mg/kg/day were associated with infertility in captive cheetahs (*95*).

Experimental studies of rats suggest that effects on ovarian cycles depend on the hormonal milieu. For example, a 0.01% dietary concentration of COM (a dose of 16 mg/kg bw/day) hastened the onset of ovarian cycles in immature females but suppressed ovarian cycles when provided to cycling adult females (*96*). Although the suppression of cycles is reversed upon cessation of treatments, prepubertal exposure may affect cyclicity later in life. Rats treated prepubertally with 500 mg/kg/day sc GEN spent more time in the estrous stage of vaginal cycles at 50 days of age (*97*), and rats treated prepubertally with 16 mg/kg/day oral COM exhibited more cycle irregularities at 100 days of age (*96*).

Several studies have examined the effects of phytoestrogen supplementation on the human menstrual cycle, with variable results (*52,53,98–101*) (Table 14). Isoflavone doses of 0.8–3 mg/kg/day produced significant changes in menstrual cycles, but the direct and type of effect varied considerably across studies. An increased length of the menstrual cycle was the most common outcome, but this change was accompanied by elevations as well as declines in serum E_2 and testosterone. The practice of sampling hormonal levels on only a few days of the cycle becomes problematical when cycle length is altered, as the days compared no longer represent comparable stages of the cycle. A recent study reported consistent reductions in urinary estrogens but minimal changes in plasma estrogens, suggesting that urinary steroids may be more sensitive indices of changes in steroid production than plasma concentrations (*100,102*). Two studies have reported declines in periovulatory luteinizing hormone (LH) and follicle-stimulating hormone (*52,100*), a response with implications for fertility suggestive of

Table 10. Estimates of isoflavone bioavailability in human subjects.[a]

Isoflavone	Source	Dose (mg/kg/day)	Peak plasma	Time to peak	Absorption half-life	Excretion half-life	Bioavailability	Ref
GEN	Soy milk	0.3–0.8					5–11%	(*196*)
GEN	Soy milk	0.3–0.8					10–37%	(*198*)
GEN	Kinako	0.4	2.4 µM	6 hr		8.4 hr	20%	(*199*)
GEN	Tofu	0.6					13–16%	(*195*)
GEN	Soy flour	1.0	4.1 µM	8.4 hr		4.7 hr	22%	(*200*)
GEN	Soy milk	1.7			2.2 → 1.4	3.8 hr	15%	(*201*)
GEN	Soy milk	1.8			2.7 → 2.0	6–8 hr	24% → 14%	(*99*)
DAI	Soy milk	0.4–1.2					16–32%	(*198*)
DAI	Soy milk	0.4–1.2					20–24%	(*196*)
DAI	Tofu	0.4					49%	(*195*)
DAI	Kinako	0.4	1.6 µM	6 hr		5.8 hr	56%	(*199*)
DAI	Soy flour	0.8	3.1 µM	7.4 hr		5.7 hr	62%	(*200*)
DAI	Soy milk	1.5			1.6 → 1.4	4–6 hr	66% → 45%	(*99*)
DAI	Soy milk	1.7			1.5 → 2.5	2.9 hr	47%	(*201*)

[a]Estimates of isoflavone bioavailability following ingestion of a soy-based product; dose gives the estimated intake in milligrams per kilogram body weight per day.

Table 11. Isoflavone absorption and excretion in animals.

Isoflavone	Species	Dose (mg/kg/day)	Plasma peak	Time to peak (hr)	Plasma half-life	Bioavailability (%)	Ref
GEN, sc	Rat	500	24 hr: 4.2 µM				(*110*)
GEN, oral	Rat	20	11 µM	2	8.8 hr	20	(*202*)
Soy genistin, oral	Rat	20	4.9 µM	2	8.8 hr	18	(*202*)
Soy GEN, oral	Rat	20	9.5 µM	2		15	(*203*)
GEN, oral	Rat	45	2.2 µM	2			(*112*)
GEN, oral	Rat	~40	6–8 µM		3–4 hr		(*77*)
Soy daidzein, oral	Rat	21	5 µM	2		19	(*203*)
EQL, sc	Rat	5	1 hr: 0.4 µM				(*76*)
GEN, iv	Mouse	52	237 µM	0.6–1.3			(*204*)
GEN, oral	Mouse	45	2.6 µM free	0.3	4.8 hr	20	(*204*)
GEN, oral	Mouse	54–180	4.1 µM free	0.05	4.7 hr	21	(*204*)
Genistin, oral	Mouse	50	1.5 µM free	0.5	8 hr > 0.4 µM	11	(*204*)
FRM, sc	Mouse	40	9.2 µM	2.5			(*205*)
GEN, oral	Rhesus macaque	7	55 nM (free + sulfate)				(*82*)
DAI, oral	Rhesus macaque	3	21 nM (free + sulfate)				(*82*)
FRM, oral	Sheep	84	0.1 µM; 7 µM equol	1			(*205*)
FRM, oral	Cow	(15 g)	0.4 µM; 7 µM equol	1			(*205*)
FRM, oral	Pig	(0.9 g)	3.7 µM; 0.9 µM equol	1			(*205*)

pituitary or hypothalamic actions of phytoestrogens. However, sample size and duration of treatment limit the conclusions that can be derived from these studies. The effects observed after only a single cycle of treatment may not be representative of the longer-term consequences of phytoestrogen consumption. Larger samples of women observed over longer periods of treatment with controlled doses are needed to establish the effects of soy isoflavones on the menstrual cycle.

Proliferation of Breast Epithelium

Low rates of breast cancer in populations with high phytoestrogen exposure suggest phytoestrogens may inhibit epithelial cell proliferation (*9,65,103–106*). On the other hand, the biphasic effects on proliferation observed in *in vitro* studies suggest that both proliferative and antiproliferative effects might be observed, depending on the dose. Results from multiple *in vivo* studies across species have been contradictory. A variety of effects have been noted, ranging from antiproliferation through enhanced proliferation, depending on the tumor cell type, dose, timing of phytoestrogen exposure, and the phytoestrogen given. In general the isoflavones, and particularly GEN (likely due at least partially to its tyrosine kinase-inhibiting properties), produce the most significant results and are thus the most widely explored.

Results seen *in vitro* have been difficult to reproduce *in vivo*. A recent study demonstrated that GEN (20 μM) inhibits cell proliferation in estrogen-independent human breast cancer cells (MDA-MB-231) by as much as 50%. Mice with breast tumors produced by inoculation with these same cells were then placed on a GEN-rich diet (750 μg/g). No significant changes in tumor size or morphology were seen, even when the treatment diet was given before the initial innoculation with the MDA-MB-231 cells (*107*).

Soy protein and GEN reduce tumor multiplicity, but not incidence, in other animal models of breast cancer (Table 15). Soy protein isolate-containing diets providing oral doses of 2–8 mg/kg/day GEN and 1–3 mg/kg/day DAI reduced the number of mammary tumors in 7,12-dimethylbenz[*a*]-anthracene (DMBA)-treated rats (*108*). GEN or DAI intraperitoneal (ip) injections that provided doses ranging from 8 mg/kg/day at 35 days of age to 3 mg/kg/day at 215 days of age resulted in an observable but nonsignificant reduction in the number of mammary tumors in *N*-methylnitrosourea (MNU)-treated rats (*109*). The effect of DAI was delayed compared with that of GEN. No significant changes in protein tyrosine kinase-mediated phosphorylation or topoisomerase II activity were observed in the mammary gland or mammary tumors, even with higher isoflavone doses (40 mg/kg/day), suggesting that the observed chemoprevention was mediated via other mechanisms (*109*). Three-day sc treatments with a much higher GEN dose (500 mg/kg/day) during the neonatal (*110*) or prepubertal period (*97*) resulted in more marked reductions (50%) in tumor number in DMBA-treated rats.

Stimulation of mammary tissue has been seen in FRM-treated mice (*111*), GEN-treated rats, (*97,112*), and in cattle grazing on phytoestrogen-rich clover (*113*) (Table 15). Five-day treatment with sc doses of 40 mg/kg/day FRM, resulting in peak plasma and mammary levels of 9 μM and 4 nmol/g, increased mammary gland proliferation in ovariectomized mice (*111*). Oral doses of 36 mg/kg/day GEN maintained lobulo-alveolar structure in ovariectomized rats without affecting ducts; no effects were seen in immature rats (*112*). Transitory increases in mammary gland weight also were produced

Table 12. Tissue and fluid concentrations of phytoestrogens.

Species	Isoflavone	Dose (mg/kg/day[a])	Peak plasma (nM)	Fluid/tissue	Concentration	Ref
Mouse	FRM, sc	40	9,200	Mammary	7 nmol/g	(*205*)
Rat	EQL, sc	5	400	Uterus	3 nmol/g	(*76*)
Rat	GEN, oral	~8	600–900	Mammary	0.3 nmol/g	(*77*)
				Uterus	0.8 nmol/g	
				Prostate	0.8 nmol/g	
		~40	6,000–8,000	Mammary	0.8 nmol/g	
				Uterus	1.4 nmol/g	
				Prostate	1.1 nmol/g	
Human - United States	GEN soy diet	0.07	100	Breast milk	30 nM	(*78*)
		0.1	350		45 nM	
		0.3	1,300		70 nM	
Human - United States	DAI soy diet	0.07	60	Breast milk	15 nM	(*78*)
		0.1	500		32 nM	
		0.3	1,400		60 nM	
Human - Lisbon	DAI	Norm. diet	5	Prostatic fluid	18 nM	(*80*)
Human - Hong Kong	DAI	Norm. diet	123	Prostatic fluid	275 nM	(*80*
Human - Hong Kong	EQL	Norm. diet	16	Prostatic fluid	709 nM	(*80*
Human - Australia	EQL	Unknown	158			(*206*)
Human - Australia	DAI	Unknown	1,147			(*206*)
Human - Australia	GEN	Unknown	544			(*206*)

Norm diet, normal diet.
[a]Human mg/kg/day estimated based on a body weight of 60 kg.

Table 13. Proliferative responses of uterine and vaginal epithelium to phytoestrogen treatments.

End point	Dose (mg/kg/day) Soy isoflavone	FRM	GEN	BIA	COM	IPR	α-ZAL	Ref
Uterine growth								
Immature mouse, sc					0.1–10			(*165*)
Immature mouse, oral		300–800	100–400	200–800	2–10			(*174*)
Immature/ovx rat, sc			0.5			200		(*97,150*)
Ovx rat, sc						Inactive		(*150*)
Immature rat, oral					16–160			(*207*)
Ovx rat, oral	Inactive 58–590/1,800 calories		10–36		1–30		0.1–30	(*83,112,142*)
Ovx lact rat, oral			Inactive 2–17					(*144*)
Ovx heifers, oral	67 FRM + 40 BIA							(*113*)
Vaginal cell maturation								
Ovx rat, oral	Inactive 58–590/1,800 calories							(*83*)
Ovx longtail macaques	10 Inactive							(*82*)
Human postmenopausal	2.8 Inactive							(*84*)
Human postmenopausal	1–2							(*85*)
Human postmenopausal	1–2 Inactive							(*86*)

BIA, biochanin A.

Table 14. Phytoestrogen influences on the human menstrual cycle.

Supplement	Dose (mg/kg/day)	Duration	*n*	Hormonal sampling	Cycle length	E_2	Testosterone	P_4	LH	Ref
Flax ENL, END	~0.09	3 cycles	18	Monthly; composite cycle	Prolonged luteal		↑			(*98*)
Soy GEN, DAI	0.75 GEN: 0.3 DAI: 0.4	1 month	6	Daily	Prolonged follicular	↑			↓	(*52*)
Soy GEN, DAI	3 GEN: 1.5 DAI: 1.5	1 month	6	Cycle days 7 and 22	Prolonged 5/6, short 1/6	↓	↓	↓		(*99*)
Soy GEN, DAI	1 GEN: 0.6; DAI:0.4	6 months	24	Random	No change	↑				(*53*)
Soy GEN, DAI	1	3 cycles	14	Every other day	No change				↓	(*100*)
	2				No change	↓ E_1 uE_2 uE_1				(*102*)
Soy milk GEN, DAI	1.5	2 cycles	31		Prolonged	E_1 ↓ $p = 0.07$				(*101*)

Abbreviations: P_4, progesterone; uE_1, urinary estrone; uE_2, urinary estradiol.

Table 15. Isoflavone actions in mammary tissue and breast cancer.

Species	Focus	Agent	Dose (mg/kg/day)	Age	Effect	Ref
Ovx mouse	Mammary epithelium	FRM	40 sc	Adult	Proliferation	(*111*)
Ovx rat	Mammary lobular alveolar structure	GEN	36 oral[a]	Adult	Maintained structure	(*112*)
Immature rat	Mammary size	GEN	500 sc	Adult	Increased weight	(*97*)
Ovx long-tail macaque	Mammary epithelium	Soy isoflavone	10 oral	Adult	No effect	(*116*)
Human	Mammary epithelium Nipple aspirate fluid	Soy isoflavone	1 oral	PRE	Proliferation ↑ Fluid	(*53*)
Human	Lobular epithelium	Soy isoflavone	0.75 oral	PRE	Proliferation; ↑ PR	(*124*)
Rat	DMBA-induced mammary cancer	GEN + DAI	GEN 2–8 DAI 1–3 oral	PND 25–155	20–40% ↓ tumor number	(*108*)
Rat	MNU-induced mammary cancer	GEN	3–8 ip	PND 35–215	27% ↓ tumor number; $p < 0.07$	(*109*)
Rat	MNU-induced mammary cancer	DAI	3–8 ip	PND 35–215	27% ↓ tumor number; $p < 0.26$	(*109*)
Rat	DMBA-induced mammary cancer	GEN	500 sc	PND 16,18,20	50% ↓ tumor number	(*97*)
Rat	DMBA-induced mammary cancer	GEN	500 sc	PND 2,4,6	50% ↓ tumor number	(*110*)
Rat	Implanted tumor cells	Soy extract	(18 mg ip)		↑ tumor growth	(*114*)
Rat	Implanted tumor cells	GEN	11 oral[a]	PND 28	↑ mammary & tumor size	(*115*)
Rat	Spontaneous tumorigenesis	ZLN	10 sc	PND 7,14	↑ tumor incidence	(*121*)

PND, postnatal day.
[a]Estimated from dietary concentration.

with three sc doses of 500 mg/kg/day GEN in prepubertal and neonatal rats (*97*). Moreover, soy isoflavones or oral GEN actually increased the growth of implanted MAC-33 or MCF7 tumor cells in some recent experiments in rats (*114,115*). However, soy isoflavones did not increase mammary epithelium proliferation in surgically postmenopausal long-tailed macaques (*116*).

Early exposure to estrogen also may influence susceptibility to mammary cancer by altering the proliferation and differentiation of epithelial structures that are sensitive to transformation by carcinogens. Neonatal exposure to E_2 stimulates the mammary epithelium in rodents, increasing terminal end bud proliferation and reducing the differentiation of terminal end buds during the period from 4 to 16 weeks of age (*117*). This effect is hypothesized to enhance susceptibility to carcinogenesis, as the terminal end bud is the site for transformation in the rodent mammary gland (*118*). Perinatal treatment with E_2 or diethylstilbestrol (DES) increases the incidence of mammary tumors in mice carrying a mammary tumor virus (*119,120*). Similar effects have been observed with phytoestrogens. Neonatal treatment with 10 mg/kg/day zearalenone (ZLN) increases the incidence of spontaneous mammary tumors in Wistar rats (*121*). Increased numbers and reduced differentiation of terminal end buds have been observed in mice after *in utero* exposure to ZLN (*117*).

Neonatal or prepubertal exposure to GEN, however, appears to have the opposing effect of enhancing terminal duct differentiation in rats. Neonatal or prepubertal doses of 500 mg/kg/day GEN significantly reduced the number of terminal end buds and cell proliferation indices at 50 days of age (*97,110*). Transforming growth factor (TGF)-α and epidermal growth factor (EGF) receptor were upregulated in terminal duct structures immediately after treatment, but the EGF signaling pathway was downregulated by 50 days of age (*122*). These data suggest that high-dose GEN enhances epithelial differentiation. However, in the absence of data on mammary structure beyond 50 days of age, it is difficult to evaluate whether the observed changes represent permanent changes in mammary organization or simply alterations in the timing of developmental events. Moreover, these high-dose treatments induced changes in the reproductive tract that resemble the alterations in sexual differentiation produced by perinatal DES or E_2. Both prenatal and neonatal GEN treatments resulted in precocious vaginal opening, and neonatal treatments were associated with reductions in serum progesterone at 50 days of age and follicular abnormalities such as atretic antral follicles and fewer corpora lutea (*123*).

Only two studies have directly examined isoflavone influences on the breast in women (Table 15). Chinese and Japanese women had lower nipple aspirate fluid volume, lower mean levels of gross cystic disease fluid protein, fewer hyperplasic cells, and fewer atypical epithelial cells in their nipple aspirate fluid than American women, an effect that was postulated to be a consequence of consumption of soy isoflavones (*53*). However, supplementation of the diets of American women with soy protein, providing GEN and DAI doses of 0.6 and 0.4 mg/kg/day, respectively, stimulated breast epithelial cell proliferation and 2- to 6-fold increases in nipple aspirate fluid volume in 29% of premenopausal (PRE) women (*53*). Monthly plasma collections suggested that E_2 also was erratically elevated. A second study examined the proliferation rate of breast epithelium in biopsy samples from women with benign or malignant breast disease treated with and without a soy supplement providing an isoflavone dose of 0.75 mg/kg/day (*124*). After 14 days of treatment, the proliferation rate of the lobular epithelium and PR expression were significantly increased when day of menstrual cycle and patient age were taken into account. These unexpected results illustrate the difficulties of predicting human responses and the need for careful clinical trials.

Prostate and Testes

Evidence for high expression of ERβ in the prostate suggests that it could be an important target for phytoestrogen action. There is evidence for estrogenic action of isoflavones in the prostate. Isoflavonoids induce c-*fos* expression in the murine prostate at doses of 0.025–2.5 mg/kg/day sc (GEN) and 5 mg/kg/day (COM, DAI) (*125,126*). Ten days of treatment with GEN (2.5 mg/kg/day sc) induced neoplastic transformation in neonatally estrogenized mice, whereas a sc dose of 1.2 mg/kg/day had no effect (*125*). On the other hand, rats maintained on a soy-free diet for 11 weeks developed prostatitis in the lateral lobe of the prostate, an inflammation that can be induced by estrogen (*127*), suggesting that soy isoflavones might antagonize the actions of endogenous estrogens in the prostate. A recent study provided evidence for a suppressive effect of isoflavones on the hypothalamic–pituitary–gonadal axis, showing that 9 days of treatment with sc GEN at a dose of 2.5 mg/kg/day reduced testicular and serum testosterone, pituitary LH content, and prostate weight in adult male mice (*126*).

GEN inhibited the growth of prostate cancer tissue in 3-dimensional histoculture at concentrations of 4–37 μM (*128*), but dietary doses of 0.07–0.3 mg/kg/day GEN had no effect on the growth of matairesinol (MAT) LyLu prostate cancer cells implanted sc in male rats (*129*). Intraperitoneal doses of 0.1–0.4 mg/kg/day also had minimal effect (*129*). However, a diet containing a high isoflavone soy protein isolate reduced tumor incidence and prolonged the latency to tumor development in MNU-treated Lobund-Wistar rats (*130*).

There are no published tests of preventative actions of phytoestrogens in human prostate cancer. However, a single clinical case has been reported in which apoptosis typical of high-estrogen therapy was seen in a man who took 160 mg of phytoestrogen per day for 1 week prior to radical prostatectomy (*131*).

Bone Density

Estrogen is required for maintenance of normal bone density, and some phytoestrogens have been investigated as potential estrogen replacement therapy in postmenopausal women. Ipriflavone, (IPR; 7-isopropoxyisoflavone), an isoflavone derivative, has been the phytoestrogen most studied as a therapeutic agent in osteoporosis. Daily oral doses of 3–10 mg/kg/day IPR prevent bone turnover and loss in bone density in women who are postmenopausal or who have been treated with gonadotropin-releasing hormone (GnRH) agonists (*132–134*). These doses produce plasma concentrations (*135*) similar to the *in vitro* concentrations (≥ 100 nM) required for inhibition of osteoclast differentiation (*136*) and bone resorption (*137*). Much higher oral doses (50–400 mg/kg/day) of IPR have been required to prevent bone loss in ovariectomized rats (*138*), doses that also produce plasma concentrations similar to the effective *in vitro* concentrations (*139,140*). COM also inhibits bone resorption *in vitro* (*141*) and is a more potent agent, preventing bone loss at oral doses of 1–2 mg/kg/day (*142,143*). Results with GEN, on the other hand, have been inconsistent. No effect on bone density is seen in ovariectomized rats with oral doses of 0.1–30 mg/kg/day GEN (*142*), whereas doses of 2 mg/kg/day (but not 6–20 mg/kg/day) preserve bone density in ovariectomized lactating rats (*144*). A dietary supplement providing approximately 10 mg/kg/day GEN, 17 mg/kg/day FRM, and 19 mg/kg/day biochanin A also had no effect on bone density (*142*). DAI, in contrast, actually stimulates bone resorption *in vitro* at concentrations of only 0.01–0.1 nM (*145*) but has not been tested *in vivo*. DAI is one of the metabolites of IPR, and its opposing effects may help to explain why such high doses of IPR are required to prevent bone loss.

The role of estrogen receptors in these actions is unclear. Although both ERα and ERβ are expressed in human (*146*) and rat (*147*) osteoblasts, there is no convincing evidence for either in osteoclasts (*148*), and preosteoclastic cells have not been examined. Unlike other isoflavonoids, IPR does not displace E_2 binding in MCF7 cells or induce ERα-dependent gene transcription (*149*). IPR enhances E_2 binding to preosteoclasts, but its binding to the same cells (K_d = 68 nM) is not displaced by E_2 (*149*). Moreover, IPR appears to have minimal or no estrogen action on its own, although it augments the estrogenic effect of E_2 and estrone (E_1) on uterine weight and thyroid calcitonin release (*150*).

Cardiovascular Function

Reductions in serum cholesterol were produced in ovariectomized rats by 4 days of oral treatment with α-zearalanol (ZAL) (ED_{50} = 0.2 mg/kg/day), COM (ED_{50} = 0.4 mg/kg/day), and GEN (ED_{50} = 0.5 mg/kg) (*142*). One month of treatment with 100 mg/kg/day IPR also reduced serum cholesterol in ovariectomized rats (*151*). A soy supplement providing approximately 7 mg/kg/day GEN plus 3 mg/kg/day DAI to ovariectomized female rhesus macaques with diet-induced atherosclerosis produced free plasma concentrations as high as 20 nM GEN and 40 nM DAI (*152,153*). The isoflavone diet improved a number of cardiovascular risk factors such as lower total plasma cholesterol, elevated high-density lipoprotein cholesterol, lowered arterial lipid peroxidation, and enhanced dilator response of atherosclerotic arteries (*152–154*).

Five- to 12-week treatments with soy supplements providing 0.7–1.5 mg/kg/day isoflavones did not alter serum lipids in men and women with average serum cholestrol concentrations (*86,155–157*), although reductions in LDL cholesterol have been observed in hypercholesterolemic women following soy isoflavone treatment (*158*). However, arterial compliance, a measure of arterial elasticity, was improved in menopausal women after 10 weeks of treatment with 0.7–1.2 mg/kg/day clover isoflavones (*157*).

Hypothalamic and Pituitary Feedback

Several studies have examined the influence of phytoestrogens on basal LH or LH release (Table 16). In ovariectomized rats, a low GEN dose (1 ng/kg iv) enhanced GnRH-induced LH release, whereas higher doses (1–10 μg/kg iv and 0.1–10 mg/kg iv) inhibited LH release (*159,160*). Pretreatment with GEN 8 mg/kg sc or ZLN (0.8–8 mg/kg sc) did not inhibit tonic LH release, but these doses did block GnRH-stimulated LH release (*161*). However, oral doses of 0.1–10 mg/kg GEN, administered by gavage, had no effect on tonic LH (*160*). In rhesus macaques, ZLN suppressed LH at sc doses of 5–14 μg/kg and oral doses of 400 μg/kg (*162*).

Table 16. Phytoestrogen effects on anterior pituitary hormones.

Species	Phytoestrogen	Dose (mg/kg/day)	Hormone	Outcome	Ref
Rhesus macaques	ZNL	0.005–0.014 sc	Plasma LH	↓ LH	(*162*)
		0.4 oral		↓ LH	
Ovx rats	ZNL	0.8–8 sc	Plasma LH	No effect	(*161*)
Ovx rats	ZNL	0.8-8.0 sc	GnRH-stimulated LH	↓ LH	(*161*)
Ovx rats	GEN	0.1–10 gavage	Plasma LH	No effect	(*161*)
Ovx rats	GEN	8 sc	GnRH-stimulated LH	↓ LH	(*159*)
		0.001–10 iv		↓ LH	(*160*)
		1 ng/kg		↑ LH	(*161*)
Ovx mice	GEN	40 mg/kg sc	Plasma PRL	↑ PRL	(*211*)
Ovx rats	GEN	36 mg/kg oral[a]	Plasma PRL	↑ PRL	(*112*)
Ovx rats	GEN	36 mg/kg oral[a]	E_2-stimulated PRL	No effect	(*112*)
Postmenopausal women	Soy isoflavones	0.7–1	Periovulatory LH	↓ LH	(*52,100*)
Postmenopausal women	Soy isoflavones	0.7–1	LH response to GnRH	↓ LH	(*163*)

[a]Estimated from dietary concentration.

There is some evidence that phytoestrogens can influence human LH secretion. Soy supplements providing 0.7–1 mg/kg/day isoflavones reduced mid-cycle LH (*52,100*), and suppression of the LH response to GnRH was observed in postmenopausal women with a similar supplement (*163*). ZLN suppressed LH secretion in postmenopausal women at similar dietary doses (0.4 mg/kg/day) (*164*).

Developmental Effects

The developmental actions of phytoestrogens have been studied mostly in rats and interpreted to have implications for human health. The impact of phytoestrogens on human fetal development is unclear, but the results of DES exposure have shown that early estrogen exposure can have profound effects. Although the immediately observable effects are limited in rodents, prenatal or neonatal exposure to phytoestrogens results in altered prepubertal or adult morphology and/or function in the uterus, vagina, ovary, breast, pituitary, and hypothalamus (Table 17). Reduced responsiveness to estrogen is apparent in the uterus, breast, and prostate, whereas symptoms of hyperestrogenization are apparent in the vaginal tract. Although the phytoestrogens vary in effective dose range and end points effected, COM, GEN, EQL, and ZLN all appear to exhibit some of the developmental actions that have been reported for other more potent nonsteroidal estrogens like DES. Active sc doses in neonates range from 0.005 to 50 mg/kg/day for COM and 10 to 500 mg/kg/day for GEN and ZLN. Effective maternal doses range from 0.2 to 17 mg/kg/day for both oral and sc doses. Although many of these doses are quite high, reports of effects at rather low doses, particularly in rat dams, argue for a fuller examination of dose–response relationships.

Summary and Conclusions

Binding studies using purified receptor proteins show that the isoflavonoid phytoestrogens are high-affinity ligands for ERs, especially ERβ. In whole-cell assays, however, their RP is significantly lower, probably as a result of interactions with binding proteins and other as yet unidentified factors. As with other endocrine-active compounds, a wide variety of enzymatic actions have been reported for phytoestrogens. However, many of these *in vitro* actions require concentrations higher than those normally seen in plasma.

In vivo data show that phytoestrogens have a wide range of biologic effects at doses and plasma concentrations seen with normal human diets. Significant *in vivo* responses have been observed in animal and human tests for bone, breast, ovary, pituitary, vasculature, prostate, and serum lipids. These actions represent a broader range of tissues and processes, including many beneficial outcomes, than the end points generally used to assess health risks posed by exposure to endocrine-active compounds (*5*). Reports of ERβ in many of the tissues that appear to be responsive to phytoestrogens are intriguing, but currently there are not sufficient data to assess the relative sensitivity of different end points. For some of these end points, similar responses to phytoestrogens have been reported in humans and animal models, although there are currently insufficient data to carry out detailed comparisons.

The biphasic actions of phytoestrogens complicate the process of assigning lowest observed adverse effect levels. In this case, the actions presumed to be more beneficial (e.g., antiproliferative actions) occur at higher *in vitro* concentrations (micromolar) than the proliferative actions presumed to be more harmful (nanomolar concentrations). This pattern would predict that higher, rather than lower, doses would be more beneficial *in vivo*. Moreover, human plasma and tissue concentrations are primarily in the nanomolar range, suggesting that proliferative effects should be more likely to be observed.

The *in vivo* situation is more complex, however. Oral soy isoflavone doses of 3–8 mg/kg/day delay the development of carcinogen-induced breast tumors in rats, whereas GEN doses reported to stimulate growth of breast and uterine epithelia range from 10 to 36 mg/kg/day. However, a recent study has shown that oral GEN doses as low as 11 mg/kg/day enhance the growth of implanted mammary tumors as well as the mammary gland. Moreover, two studies have reported proliferation of breast epithelium in women with oral soy isoflavone doses of 0.7–1 mg/kg/day. The similarity of

Table 17. Developmental effects of phytoestrogens and mycoestrogens in rodents.

Tissue	Species	Life stage	Phytoestrogen	Dose (mg/kg/day)	Effect	Ref
Uterus	Rat	Neonatal	COM	1–10 sc	Uterine and gland growth, later ↓ weight, ER	(*212,213*)
			COM	25–50 sc	Squamous metaplasia	
			EQL	10–100 sc	Later ↓ weight	
			ZNL	1–100 sc	Uterine growth, later?	
Vagina	Mouse	Neonatal	COM	0.005–50 sc	Persistent cornification	(*214,215*)
			COM	25–50 sc	Cervicovaginal adenosis	
	Rat	Prenatal	GEN	17 sc - dams	Delayed vaginal opening, normal cycles	
Ovary	Mouse	Neonatal	COM	10 sc	Polyovular follicles	(*215*)
	Rat	Neonatal	GEN	500 sc	Atretic antral follicles, ↓ corpora lutea	(*123,216*)
		Prenatal, neonatal	GEN	0.2 oral - dams	Atretic follicles, cystic rete ovarii	
Breast	Rat	Neonatal	GEN	500 sc	Increased terminal duct differentiatiion	(*123*)
			ZNL	10 sc	Spontaneous tumors	
Anogenital	Rat	Prenatal	GEN	17 sc - dams	Decreased distance in males	(*214*)
Prostate	Mouse	Neonatal DES	COM, DAI	7 sc - adults	Increased c-*fos*	(*217*)
		Neonatal DES	13% Soy diet	[9.4 μM excreted/day]	Prevention of DES lesions	
Pituitary	Rat	Neonatal	COM	0.01–1 sc	↑ Basal LH in females	(*218,219*)
			COM	1 sc	↓ GnRH-stimulated LH	
			GEN	10 sc	↑ GnRH-stimulated LH	
			GEN	100 sc	↓ GnRH-stimulated LH, ↓ basal LH	
			ZNL	10–100 sc	↓ GnRH-stimulated LH, ↓ basal LH	
Hypothalamus	Rat	Neonatal	COM	7 oral - dams	Premature anovulation, abnormal sexual behavior in males	(*220*)
SDN-POA	Rat	Neonatal	COM	0.01–1 sc	No effect	(*218,219*)
			GEN	100 sc	No effect (10), enlarged in females (100)	
			ZNL	100 sc	Enlarged in females, 10: no effect	

SDN-PDA, sexually dimorphic nucleus of preoptic area.

reported proliferative and antiproliferative doses illustrates the need for fuller examination of dose–response relationships and multiple end points in assessing phytoestrogen actions.

The lowest effective concentrations in animals have been reported for induction of c-*fos* in the prostate and regulation of cholesterol, testosterone, and LH. The low effective doses reported for c-*fos* suggest that molecular or biochemical end points may be more sensitive indices of phytoestrogen response than the morphologic or functional end points commonly tested. The low doses reported for phytoestrogen effects on hormonal secretion suggest that steroidogenesis and the hypothalamic–pituitary–gonadal axis are important loci of phytoestrogen actions. However, these inferences must be tentative because good dose–response data are not available for many end points and many of the doses may not be the lowest active doses.

The available data indicate that phytoestrogens are biologically active in humans at dietary doses of 0.4–10 mg/kg/day (Table 18). The active isoflavone doses are similar to the daily intakes estimated for adults consuming soy-rich Asian diets, which may be primarily a consequence of attempts to test the hypothesized health benefits of Asian diets. Few human studies have attempted to test a range of doses. Moreover, all of the human studies have used isoflavone mixtures present in extracts of soy or red clover, making it difficult to assess the contributions and activity of individual isoflavones. This issue is particularly important in light of the *in vitro* and *in vivo* data showing that GEN and DAI sometimes exhibit opposing effects. Moreover, most studies have relied on manufacturer's data on isoflavone content to estimate daily intake, so the actual doses used may vary even in studies using the same soy supplements.

The doses reported to be active in humans are lower than the doses generally reported to be active in rodents (10–100 mg/kg/day), although some studies have reported rodent responses at lower doses (0.005–0.2 mg/kg/day). However, the available estimates of bioavailability and peak plasma levels in rodents and humans are more similar. More studies are needed to determine whether lower doses are effective in rodents and humans and whether bioactive doses are associated with similar plasma levels in humans and animal models.

These comparisons illustrate the rich database available on phytoestrogen actions, mechanisms, and metabolism and highlight gaps and discrepancies in the dataset. These findings demonstrate some of the complexities of extrapolating across assays, species, and compounds. As dietary components, phytoestrogens generally have been treated and tested like nutrients rather than pharmacologic agents. As a result, researchers have investigated a number of beneficial properties that would be overlooked in toxicologic studies but also have failed to anticipate some potentially adverse properties. There is currently very little information on the balance of effects in individuals, data that ultimately will be required in order to assess the wisdom of phytoestrogen consumption. More detailed pharmacokinetic data are required to more accurately evaluate the reliability of extrapolating from *in vitro* to *in vivo* studies or across species. Such comparisons are likely to provide new insights into the evaluation of other exogenous estrogens.

Table 18. Phytoestrogen actions in humans and animal models.[a]

Tissue	End point	Isoflavone dose (mg/kg/day)	
		Rats	Humans
Reproductive tissues			
Uterine endometrium	Proliferation	10–36	No effect of soy
Vaginal cytology	Cornification	16	Variable
Ovarian function	Ovarian hormones	16	0.7–3 oral
Breast epithelium	Proliferation	36–500	0.75–1 oral
Terminal ducts	Differentiation	500	Untested
Breast tumor	Suppression	3–11	Untested
Prostate	Growth	2.5	Untested
	c-*fos* induction	0.025–5	Untested
Testes	Reduced testosterone	2.5	Untested
Skeletal			
Bone	Increased density	50–400 variable	3–10 oral
Cardiovascular			
Cardiovascular function	Reduced LDL cholesterol	0.2–0.5	0.7–1 oral, only in hypercholestemic
Neuroendocrine			
Hypothalamic, pituitary feedback	Reduced LH	0.1–10 sc	0.7–1 oral
Developmental effects	Gonadotropin secretion	0.005–500 sc	Untested
	SDN-POA	7–17 maternal	Untested
	Anogenital distance	exposure, sc	Untested
	Uterine and vaginal development	Maternal	Untested

[a]Adapted from Whitten and Naftolin (*221*).

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